

July 18, 1973

Dear Dr. Kosin

Here is the basic procedure I promised long ago. It may not be complete for the information you need, but I hope it is a beginning.

I have tried to give you the evolution of how this technique was developed, and as I was writing one thing, I would think of something else so I also wrote that down so as not to forget. I just hope it does not sound too jumbled up for you to understand.

I have not included a source list for materials. As I think of the various materials, I will write them down and list their source. If you need any information of that type, please write and let me know.

FIXATION OF EGG BLASTODERM:

The basic fixation procedure is to use and 8% glutaraldehyde solution which is diluted to 8% from a 50% solution sold by Electron Microscope Sciences.

The 8% solution is made up in a 0.1M collidine buffer.

Other dilutions of glutaraldehyde were tried(3%,6%) and also the use of a phosphate buffer. The 8% appeared to give the fastest penetration through the vitline membrane. Also ,no difference could be found in cell detail between the collidine and phosphate buffers, but the collidine appeared to help in the faster penetration of the glutaraldehyde fixative.

The best method for fixation would be to have the fixative penetrate from both sides of the blastoderm, and in early trials fixations this method was tried; the fixative was injected very carefully under the blastoderm with a small needle and syringe. It was found that even slight disruptions of the vitline membrane and the flow of fixative under the blastoderm, would cause the cells to loose adherence and float free. After many trials, this method was abandoned in hopes of trying to get good penetration from the outside of the vitline membrane.

Because the albumin covering the vitline membrane is a good adhesive, small rings were applied to this surface and filled with the fixative. Because of the depth of the albumin the fixative penetration was very poor, so it was decided to try and wipe some of the albumin off from the egg surface. This method was also not entirely successful causing deformation in the blastoderm disk. Again after many trials it was found that the albumin could be removed from the vitline membrane as a sheet by slipping the round tip of a pair of surgical sissors under the albumin and making a cut. With three to four cuts around the blastoderm the albumin would slip down the side of the egg, leaving the exposed surface of the vitline membrane. The ring was now applied directly to the vitline membrane. Adherence to this surface is not quite as good as with the thick albumin coating, but with care the ring will stay well attached throughout the fixative procedure.

This procedure was developed for Fresh and eggs stored short periods of time. The longer stored eggs do require a few minor changes.

As the eggs are stored for longer time periods -greater than 3 weeks, the albumin becomes thinner and the vitline membrane more difficult to handle. It was found that by cooling these eggs down to about 6-10C before fixation that they were much easier to handle and resistant to breakage of the vitline membrane.

Fixatives were applied at both room temperature for the first 10-30 min. of total fixation and others fixed directly in the cold, with no observable differences.

In the very late stored eggs- greater than 10 week , it was almost impossible to remove the egg from the shell without first fixing the blastoderm. In these cases the fixative was put directly onto the blastoderm after the top shell was cut away. After the blastoderm was sufficiently hard, it could be cut out and removed. A ring could also be applied to facilitate handling.

It was also found necessary to remove the excess yoke from around the blastoderm in all of the eggs to help the osmium penetrate. Because the osmium is used up so fast in fixing the yoke, an excess yoke will keep the osmium from penetrating to the blastoderm cells.

After the cells were fixed in glutaraldehyde, they were resistant to mechanical stress and the wiping away of the excess yoke, did not appear to cause any damage. While observing under the binocular dissecting scope the excess yoke could be wiped away from around the blastoderm and over it with a piece of soft wiping tissue.

On page 4, you will find the basic procedure I have been following.

They told us yesterday that our cooling unit on the roof had overheated and had burned up the wiring. Our temperatures are on the rise again. I sure hope they can get it fixed soon. The temperature outside has been 90 - 100 for the last week with no break in sight at this time. I think some will start harvesting this week.

See you when it gets cool (you lucky)

Jim

Procedure for the fixation of egg blastoderm revised from 29 III 73

[Fresh and Stored eggs]

Fixation: 8% glutaraldehyde in 0.1M collidine buffer

1. Cool egg to about 6C in the refrigerator before fixation - about 1 hour
2. Break egg into small dish and orient blastoderm up with spoon if necessary. In long stored eggs, break away top of the egg shell and proceed to step 4.
3. Cut albumin around blastoderm with fine round pointed scissors and separate from the vitline membrane.
4. Apply a teflon ring over the blastoderm on top of the vitline membrane.
5. Add fixative to the ring and around the outside of the ring and seal with a piece of poly film. Put into refrigerator for 1/2 - 1 hour.
6. Cut the vitline membrane around the outside of the teflon ring with fine scissors and lift the ring, with blastoderm attached, free from the egg yoke.
7. Remove the excess egg yoke from the blastoderm by holding the ring on edge and wiping off the yoke drop. Under the dissecting scope wipe off all the excess yoke from around the blastoderm, being careful not to injure the blastoderm cells.
8. Put ring with blastoderm side up, back into fresh glutaraldehyde fixative in the refrigerator and leave for 1-2 hours more.
9. Remove the fixative and wash with cold 0.1M buffer for 5 min. Change the buffer and leave 5 min. Change again and leave in the refrigerator for about 16 hours.
10. Remove the buffer and replace with 2% osmium in 0.1M buffer and put back into the refrigerator.
11. Leave in osmium for 2-4 hours with changes of osmium as it begins to turn black or dark.
12. Rinse in buffer and bring to room temperature.
13. Dehydrate in alcohol series to propylene oxide.
14. Infiltrate with Epoxy resin to 100% Epoxy
15. Harden in oven